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Changes in Abscisic Acid Contents of Some Aquatic Plants Exposed to Cadmium and Salinity

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Abstract: The content of absicisic acid in aquatic macrophytes (*Myriophyllum heterophyllum* Michx. and *Potamogeton crispus* L.) grown in cadmium medium (0, 4, 8, 16, 32 and 64 mg L^{-1}) and cadmium+salt (0, 0.05 and 5‰ NaCl) was investigated. In *Potamogeton crispus*, the content of abscisic acid was found higher than *Myriophyllum heterophyllum* before treatment of cadmium. It was observed that the content of abscisic acid increased with increased in cadmium concentration (0, 4, 8, 16, 32 and 64 mg L^{-1}) and time period (24 and 96 h) in both of species. The content of abscisic acid increased by salinity like the treatment with Cd. When the effects in combination of cadmium and salinity were considered, it was found that the content of abscisic acid increased with increased in cadmium at 0.05‰ NaCl but decreased at 5‰ NaCl. In the both species, the highest content of abscisic acid was observed in 64 mg L^{-1} Cd +0.05‰ NaCl at 96 h.

Key words: Cadmium, abscisic acid, aquatic plants, NaCl

INTRODUCTION

Cadmium (Cd) is highly toxic to animals and plants. In plants, exposure to Cd causes reductions in photosynthesis, water and nutrient uptake. As a consequence, Cd-exposed plants show various symptoms of injury such as chlorosis, growth inhibition, browning of root tips and finally death (Padmaja *et al*., 1990; Chugh and Sawhney, 1999; Schützendübel *et al*., 2002). Cadmium was found to produce oxidative stress and cadmium ions can inhibit the activity of several antioxidative enzymes (Somashekaraiah *et al*., 1992; Aravind and Prasad, 2003).

Macroalgae have a number of morphogenic events in their life cycle (germination, branching, reproduction events and senescence) which require some level of organization. It is thus to be expected that plant growth regulators play a role in controlling some of these events as they do in higher plants (Stirk *et al*., 2003). Abscisic acid mediates several plant responses to abiotic stress (Achuo *et al*., 2006). In aquatic plants, ABA induces a distinct developmental switch, substitutes the requirement for environmental stimuli and causes a series of morphogenetic response, including cell division, expansion and differentiation, leading to the alteration of morphology in leaf, stem and root (Lin and Abrams, 2004).

It was reported that chlorophyll, ABA, organic acid contents, phenolic compounds and water potentials of the plants were shown as indicator factors of the plants exposed to heavy metal stress (Terry and Stone, 2002; Sakihama *et al*., 2002; Talarico, 2002; Sivaci *et al*., 2007).

It was observed increased abscisic acid levels by NaCl concentration in leaves of *Lycopersicon esculentum* cultivars (Yürekli *et al*., 2001). The salinity of stormwater may affect the plant growth rate and plant metal uptake through the toxic effects of both the Na^+ and Cl^- ions. Na^+ ions may release Cd from the sediment to water, thereby increasing the Cd concentration in the water. In the higher salinity water, fewer free Cd ions correlates with a lower uptake of Cd by *Potamogeton pectinatus* L. growing in like this areas (Greger *et al*., 1995).

Many studies have been published about NaCl and heavy metal stress in aquatic macrophytes (Sivaci *et al*., 2004; Fritioff *et al*., 2005). However, there are no reports on how the abscisic acid levels in *Myriophyllum heterophyllum* Michx. and *Potamogeton crispus* L. changed by the combined effects of salinity and Cd stress. Therefore, the aim of this study is to investigate the changes of abscisic acid levels related to Cd (0, 4, 8, 16, 32 and 64 mg L^{-1}) and Cd+NaCl (0, 0.05 and 5‰) absorption in macrophyte species, namely *Myriophyllum heterophyllum* and *Potamogeton crispus*.

MATERIALS AND METHODS

Plant material: This study was made in the Laboratory of Biology Department in Sinop University in 2006. The samples of *Myriophyllum heterophyllum* and *Potamogeton crispus* collected from the Sinop-Karagöl-Aksaz lagüne lake (Black Sea Region/Turkey) were washed with diluted HCl solution (3%), followed by distilled water before use (Keskinkan *et al*., 2003).

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Analytical grade cadmium sulphate was used as the metal source and stock solution was prepared in deionised water. Experiments were conducted at 25°C in conical flasks (250 mL cadmium solution at varying concentrations) using an orbital shaker (200 rpm) in a constant room temperature under 12 h light and 12 h dark. Macrophyte samples (about 1 g wet weight) were added to each flask and placed on the orbital shaker. They were exposed to cadmium solution at the initial metal concentrations 0, 4, 8, 16, 32 and 64 mg L^{-1} and NaCl 0, 0.05 and 5‰. After contacting periods (24 and 96 h), plant materials were filtered to separate the biomass from the solution for the determination of abscisic acid.

Analysis of ABA: Leave samples of 1 g were placed in 100 mL methanol: chloroform: 2 N ammonium hydroxide (12:5:3 v/v/v) and homogenized by using a Kinematic Polytron homogenizer. After addition of 1 μg/100 mL butylated hydroxytoluene (BHT), the samples were frozen at -80°C for one week, for further analysis. After incubation, extracts were transferred into 250 mL conical flasks and 22.4 mL bidistilled water was added. To obtain a homogenized mixture of chloroform, methanol, water and extract, conical flasks were shaken three or four times. Thus, except for plant growth substances, other organics in methanol were allowed to pass into the chloroform phase. Extraction, purification and quantitative determination of ABA in leaves of *M. heterophyllum* and *P. crispus* were done according to literature methods (Ünyayar *et al*., 1996; Yürekli *et al*., 1999). Absorbance of the samples was recorded at 263 nm. Total ABA contents of the samples were calculated through using the calibration curve by using ABA standard. Five standards were prepared (from 10^{-2} M to 10^{-6} M concentration) by serial dilution for each hormone. Results are the mean of three replicates.

Statistical analysis: Data from three replications of all treatments were subjected to analysis of variance using SPSS 8.0 for Windows (SPSS, Chicago, IL, USA) for all statistical analysis. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The ABA levels between initial and after treatment were compared with t-test for each species following 24 and 96 h exposure. ABA contents in *M. heterophyllum* increased significantly with increasing Cd concentrations and cadmium+salinity at 24 and 96 h ($p<0.05$). Endogenous abscisic acid level in *M. heterophyllum* at 96 h was higher than 24 h. Increase in ABA content of *M. heterophyllum* with increasing Cd concentration was significant compared to control group. While 13.97 μg g^{-1} total abscisic acid contents were determined at 4 mg L^{-1} Cd, only 7.81 μg g^{-1} was determined in control group at 24 h. Abscisic acid level was increased by combined Cd concentrations and 0.05‰ NaCl but decreased by Cd+5‰ NaCl exposure time (24 and 96 h). In *M. heterophyllum*, the highest abscisic acid content was observed at 96 h and 64 (mg L^{-1} Cd+0.05‰ NaCl (46.35 μg g^{-1}) (Table 1).

ABA contents in *P. crispus* increased significantly with increasing Cd concentrations and cadmium+salinity at 24 and 96 h ($p<0.05$). The lowest ABA endogenous content in *P. crispus* were observed in control group (11.33 μg g^{-1}) and the highest in 64 mg L^{-1} Cd+ 0.05‰ NaCl (42.36 μg g^{-1}) at 24 h (Table 2). Abscisic acid level in *P. crispus* at 96 h was higher than 24 h. As mentioned in *M. heterophyllum*, the abscisic acid level of *P. crispus* was decreased by Cd+5‰ NaCl and exposure time (24 and 96 h). The highest abscisic acid level observed in 64 (mg L^{-1} Cd+ 0.05% NaCl (44.29 μg g^{-1}) at 96 h.

In the present study, ABA contents were found to increase by increasing Cd concentration in studied macrophyte species (Table 1, 2). ABA is a plant growth regulator that has been identified as a messenger in stress-perception-response pathways (Zeevaart and

Table 1: Abscisic acid content in leaves of *M. heterophyllum* exposed to Cd and salinity for 24 and 96 h (Fresh weight)

	Abscisic acid (μg g^{-1})					
	Cd concentration (mg L^{-1})					
Treatments	0	4	8	16	32	64
24 h						
Cd	7.81±1.00	13.97±0.57	22.33±1.75	31.68±1.86	37.22±1.11	39.86±0.74
Cd+0.05‰ NaCl	8.82±0.43	15.50±1.68	25.74±1.68	33.12±2.42	39.06±0.37	40.91±0.37
Cd+5‰ NaCl	9.54±1.43	12.13±1.85	21.54±3.52	29.98±0.93	35.50±1.68	37.45±1.30
96 h						
Cd	8.35±1.71	15.52±1.34	25.86±1.42	32.55±1.67	39.00±0.14	44.00±0.93
Cd+0.05‰ NaCl	10.15±0.57	20.37±1.85	29.03±2.99	34.03±1.09	41.18±1.11	46.35±0.37
Cd+5‰ NaCl	11.98±1.30	13.86±0.86	24.49±0.55	30.36±1.30	38.00±0.05	43.20±0.49

Table 2: Abscisic acid contents in leaves of *P. crispus* exposed to Cd and salinity for 24 and 96 h (Fresh weight)

	Abscisic acid (μg g^{-1})					
	Cd concentration (mg L^{-1})					
Treatments	0	4	8	16	32	64
24 h						
Cd	11.33±1.09	18.47±1.12	23.89±0.55	28.51±4.48	35.51±0.55	40.91±1.12
Cd+0.05‰ NaCl	12.04±1.25	20.45±0.55	24.35±2.33	29.30±4.80	36.70±0.37	42.36±0.55
Cd+5‰ NaCl	14.51±0.37	17.43±1.12	21.18±1.49	26.00±1.12	33.61±2.05	38.17±0.59
96 h						
Cd	12.12±0.18	21.77±1.30	30.89±1.49	34.98±0.94	39.60±1.12	43.03±0.74
Cd+0.05‰ NaCl	14.18±0.56	25.47± 1.68	32.34±0.93	36.00±2.42	41.18±0.36	44.29±0.74
Cd+5‰ NaCl	17.56±2.80	20.57± 2.24	28.35±0.43	32.54±1.11	37.71±0.74	40.20±1.49

Creelman, 1988; Wu *et al*., 1997). In *Arabidopsis thaliana*, it was reported that cadmium was highly toxic for the plant, by leading to a strong decrease in plant growth, photosynthesis and leaf conductance as already observed in other plant species. Cd toxicity is a perturbation of plant-water relationship. Such a decrease in the leaf conductance could be explained by synthesis of ABA in response to Cd stress (Perfus-Barbeoch *et al*., 2002). ABA contents in Cd-treated *Oryza sativa* L. seedlings of two cultivars were also investigated. On treatment with $CdCl_2$, the ABA content increased rapidly in the leaves and roots of Cd-tolerant cultivar but not in the Cd-sensitive cultivar (Hsu and Kao, 2003). In another study, it was determined an increase at abscisic acid concentration with Cd treatment in *Myriophyllum* species (Sivaci *et al*., 2007). It was reported that Cd treatment led to increased ABA levels in roots. *Phragmites* showed higher ABA levels compared to *Typha*. The increase of ABA content indicates the involvement of this phytohormone in early stress responses, while the stimulation of OASTL (O-acetyl-serine (thiol) lyase) following the ABA application suggests that ABA has a role in an OASTL activation pathway (Fediuc *et al*., 2005). In another study, a decrease at cytokinin concentration was observed in Cd treated wheat seedlings (Veselov *et al*., 2003). High Cd concentrations could damage the mechanism of water uptake and ion transport due to the degeneration of root tip cells, leading to a reduction in the relative water content and cytokinin transport from the roots as well as an increase in the abscisic acid contents (Prasad, 1995).

Ünyayar (2002) reported that abscisic level in fungi was increased by salt stress. The other study comparatively evaluated the effects of salt stress on leaf relative water content, soluble protein, the phytohormones indole acetic acid, gibberellic acid, zeatin and ABA levels in *Phaseolus vulgaris* and *Phaseolus acutifolius*. Treatment with 50, 100 and 150 mM NaCl caused decrease in relative water and protein content in *P. vulgaris*, but did not affect to *P. acutifolius* in this aspect. Varietal differences between *P. vulgaris* and *P. acutifolius* were also observed in hormonal content during the stress period. ABA levels in salt-treated plants of *P. vulgaris* increased but did not change in *P. acutifolius* versus the controls (Yürekli *et al*., 2004). Jia *et al*. (2002) compared salt-stress-induced the ABA accumulation in maize root tissues with that in leaf tissues and reported that ABA accumulation increased by salt stress in root and leaf of maize. In the present study, the ABA levels in two macrophyte species were increased by salinity alone (0.05 and 5‰ NaCl) (Table 1, 2). A reason of ABA accumulation by salt stress may be induced water deficit.

The present study has indicated that abscisic acid level increased by Cd+0.05‰ NaCl and decreased by Cd+5‰ NaCl treatment in two macrophyte species (Table 1, 2). Sepehr and Ghorbanli (2006) reported that the presence of cadmium in the nutrient solution in combination with NaCl affected the responses of plants. At moderate levels of cadmium and NaCl, shoot and root growth, as well as photosynthesis were improved compared with cadmium and salinity alone. The cadmium influx into plants decreased in the presence of salinity, thus reduced the harmful effects of salinity and cadmium. Cd uptake by maize plants also decreased with increasing salinity in the medium. Fritioff *et al*. (2005) reported that heavy metal uptake by submersed plants decreased with increasing salinity in the medium. Consequently, in this study Cd uptake in two macrophyte species may be decrease by salinity and the effect of Cd stress may be recovery by salinity (Cd+5‰ NaCl).

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